# WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, July 11, 2007

| Hide? | Set Name | Query | Hit Count |
|---|---|---|---|
| | | *DB=PGPB,USPT,EPAB,JPAB,DWPI; PLUR=YES; OP=OR* | |
| ☐ | L6 | L5 and (BMPI$5 OR ACUI$5 OR THAIV$5 OR BSGI$5) | 1 |
| ☐ | L5 | L3 and (xu or kobbe or zhu or samuelson).in. | 29 |
| ☐ | L4 | L3 same (BMPI$2 OR ACUI$2 OR THAIV$2 OR BSGI$2) | 2 |
| ☐ | L3 | L2 same (cleav$5 or cataly$4 or methyl$6 or target$4 or specif$5 or recogn$6 or bind$4) | 774 |
| ☐ | L2 | L1 same (CHIME$5 OR FUS$4 OR MUTA$5 OR COMBIN$5) | 1100 |
| ☐ | L1 | RESTRICTI$4 same ENDONUCLEAS$4 same (IIg or ii-g or ii) | 4519 |

END OF SEARCH HISTORY

=> d his full

(FILE 'HOME' ENTERED AT 17:11:57 ON 11 JUL 2007)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 17:12:16 ON 11 JUL 2007

SEA RESTRICTI?(S)ENDONUCLEAS?(S)TYPE?(S)II

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62 FILE AGRICOLA
3 FILE ANABSTR
1 FILE ANTE
2 FILE AQUALINE
20 FILE AQUASCI
62 FILE BIOENG
340 FILE BIOSIS
478 FILE BIOTECHABS
478 FILE BIOTECHDS
403 FILE BIOTECHNO
139 FILE CABA
550 FILE CAPLUS
38 FILE CEABA-VTB
4 FILE CONFSCI
1 FILE DDFU
1273 FILE DGENE
44 FILE DISSABS
2 FILE DRUGU
1 FILE EMBAL
226 FILE EMBASE
248 FILE ESBIOBASE
4 FILE FROSTI
29 FILE FSTA
536 FILE GENBANK
198 FILE IFIPAT
461 FILE LIFESCI
309 FILE MEDLINE
3 FILE NTIS
4 FILE OCEAN
161 FILE PASCAL
4 FILE PROMT
240 FILE SCISEARCH
76 FILE TOXCENTER
935 FILE USPATFULL
100 FILE USPAT2
118 FILE WPIDS
1 FILE WPIFV
118 FILE WPINDEX
7 FILE NLDB

L1 QUE RESTRICTI?(S) ENDONUCLEAS?(S) TYPE?(S) II

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D RANK

FILE 'USPATFULL, CAPLUS, GENBANK, BIOTECHDS, LIFESCI, BIOTECHNO, BIOSIS, MEDLINE, ESBIOBASE, SCISEARCH' ENTERED AT 17:13:59 ON 11 JUL 2007

L2 4500 SEA RESTRICTI?(S) ENDONUCLEAS?(S) TYPE?(S) II
L3 366 SEA L2(S)(IIG? OR II(4W) G)
L4 331 SEA L3(S)(CHIMER? OR MUTA? OR COMBIN? OR FUS?)
L5 329 DUP REM L4 (2 DUPLICATES REMOVED)
L6 329 SEA L5(S)(CLEAV? OR CATALY? OR METHYLAS? OR TARGE? OR SPECIF? OR RECOGN? OR BIND?)
L7 329 FOCUS L6 1-
D TI L7 1-100
D TI L7 101-200
D TI L7 201-329
D L7 IBIB ABS 1 2 6
L8 1 SEA L7 AND (BMPI? OR ACUI? OR THAIV? OR BSGI?)
D TI L8

L9 165765 SEA (BMPI? OR ACUI? OR THAIV? OR BSGI?)
L10 3662 SEA L9(S)(METHYL? OR CATALYTI? OR SPECIFI? OR BIND? OR RECOGN?)
L11 381 SEA L10(S)(CHIME? OR FUS? OR MUTA? OR COMBIN?)
L12 3 SEA L11(S)(IIG OR II(2W) G)
D TI L12 1-3
D IBIB ABS L12 1-3

Connecting via Winsock to STN

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LOGINID:ssspta1652dmr

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

| | | |
|---|---|---|
| NEWS 1 | | Web Page for STN Seminar Schedule - N. America |
| NEWS 2 | MAR 15 | WPIDS/WPIX enhanced with new FRAGHITSTR display format |
| NEWS 3 | MAR 16 | CASREACT coverage extended |
| NEWS 4 | MAR 20 | MARPAT now updated daily |
| NEWS 5 | MAR 22 | LWPI reloaded |
| NEWS 6 | MAR 30 | RDISCLOSURE reloaded with enhancements |
| NEWS 7 | APR 02 | JICST-EPLUS removed from database clusters and STN |
| NEWS 8 | APR 30 | GENBANK reloaded and enhanced with Genome Project ID field |
| NEWS 9 | APR 30 | CHEMCATS enhanced with 1.2 million new records |
| NEWS 10 | APR 30 | CA/CAplus enhanced with 1870-1889 U.S. patent records |
| NEWS 11 | APR 30 | INPADOC replaced by INPADOCDB on STN |
| NEWS 12 | MAY 01 | New CAS web site launched |
| NEWS 13 | MAY 08 | CA/CAplus Indian patent publication number format defined |
| NEWS 14 | MAY 14 | RDISCLOSURE on STN Easy enhanced with new search and display fields |
| NEWS 15 | MAY 21 | BIOSIS reloaded and enhanced with archival data |
| NEWS 16 | MAY 21 | TOXCENTER enhanced with BIOSIS reload |
| NEWS 17 | MAY 21 | CA/CAplus enhanced with additional kind codes for German patents |
| NEWS 18 | MAY 22 | CA/CAplus enhanced with IPC reclassification in Japanese patents |
| NEWS 19 | JUN 27 | CA/CAplus enhanced with pre-1967 CAS Registry Numbers |
| NEWS 20 | JUN 29 | STN Viewer now available |
| NEWS 21 | JUN 29 | STN Express, Version 8.2, now available |
| NEWS 22 | JUL 02 | LEMBASE coverage updated |
| NEWS 23 | JUL 02 | LMEDLINE coverage updated |
| NEWS 24 | JUL 02 | SCISEARCH enhanced with complete author names |
| NEWS 25 | JUL 02 | CHEMCATS accession numbers revised |
| NEWS 26 | JUL 02 | CA/CAplus enhanced with utility model patents from China |

NEWS EXPRESS 29 JUNE 2007: CURRENT WINDOWS VERSION IS V8.2,
CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
AND CURRENT DISCOVER FILE IS DATED 05 JULY 2007.

| | |
|---|---|
| NEWS HOURS | STN Operating Hours Plus Help Desk Availability |
| NEWS LOGIN | Welcome Banner and News Items |
| NEWS IPC8 | For general information regarding STN implementation of IPC 8 |

Enter NEWS followed by the item number or name to see news on that specific topic.



* * * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * * *

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FILE 'HOME' ENTERED AT 17:11:57 ON 11 JUL 2007

=> index bioscience medicine
FILE 'DRUGMONOG' ACCESS NOT AUTHORIZED
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
       AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
       CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
       DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 17:12:16 ON 11 JUL 2007

70 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> s restricti?(s)endonucleas?(s)type?(s)ii
          62   FILE AGRICOLA
           3   FILE ANABSTR
           1   FILE ANTE
           2   FILE AQUALINE
          20   FILE AQUASCI
          62   FILE BIOENG
         340   FILE BIOSIS
         478   FILE BIOTECHABS
         478   FILE BIOTECHDS
         403   FILE BIOTECHNO
         139   FILE CABA
         550   FILE CAPLUS
          38   FILE CEABA-VTB
           4   FILE CONFSCI
           1   FILE DDFU
  22 FILES SEARCHED...
        1273   FILE DGENE
          44   FILE DISSABS
           2   FILE DRUGU
           1   FILE EMBAL
         226   FILE EMBASE
         248   FILE ESBIOBASE
           4   FILE FROSTI
          29   FILE FSTA
         536   FILE GENBANK
  35 FILES SEARCHED...
         198   FILE IFIPAT
         461   FILE LIFESCI
         309   FILE MEDLINE
           3   FILE NTIS
           4   FILE OCEAN
         161   FILE PASCAL
           4   FILE PROMT
         240   FILE SCISEARCH
          76   FILE TOXCENTER
         935   FILE USPATFULL
  60 FILES SEARCHED...
         100   FILE USPAT2
         118   FILE WPIDS
           1   FILE WPIFV
         118   FILE WPINDEX
           7   FILE NLDB

  39 FILES HAVE ONE OR MORE ANSWERS,    70 FILES SEARCHED IN STNINDEX

L1    QUE RESTRICTI?(S) ENDONUCLEAS?(S) TYPE?(S) II
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=> d rank
F1         1273   DGENE
F2          935   USPATFULL
F3          550   CAPLUS
F4          536   GENBANK
F5          478   BIOTECHABS
F6          478   BIOTECHDS
F7          461   LIFESCI
F8          403   BIOTECHNO
F9          340   BIOSIS
F10         309   MEDLINE
F11         248   ESBIOBASE
F12         240   SCISEARCH
F13         226   EMBASE
F14         198   IFIPAT
F15         161   PASCAL
F16         139   CABA
F17         118   WPIDS
F18         118   WPINDEX
F19         100   USPAT2
F20          76   TOXCENTER
F21          62   AGRICOLA
F22          62   BIOENG
F23          44   DISSABS
F24          38   CEABA-VTB
F25          29   FSTA
F26          20   AQUASCI
F27           7   NLDB
F28           4   CONFSCI
F29           4   FROSTI
F30           4   OCEAN
F31           4   PROMT
F32           3   ANABSTR
F33           3   NTIS
F34           2   AQUALINE
F35           2   DRUGU
F36           1   ANTE
F37           1   DDFU
F38           1   EMBAL
F39           1   WPIFV


=> f2-f12
F2-F12 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> file f2-f12
COST IN U.S. DOLLARS                        SINCE FILE      TOTAL
                                              ENTRY     SESSION
FULL ESTIMATED COST                            1.89        2.10

FILE 'USPATFULL' ENTERED AT 17:13:59 ON 11 JUL 2007
CA INDEXING COPYRIGHT (C) 2007 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CAPLUS' ENTERED AT 17:13:59 ON 11 JUL 2007
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FILE 'GENBANK' ENTERED AT 17:13:59 ON 11 JUL 2007

FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED
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FILE 'BIOTECHDS' ENTERED AT 17:13:59 ON 11 JUL 2007
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FILE 'LIFESCI' ENTERED AT 17:13:59 ON 11 JUL 2007
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FILE 'ESBIOBASE' ENTERED AT 17:13:59 ON 11 JUL 2007
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FILE 'SCISEARCH' ENTERED AT 17:13:59 ON 11 JUL 2007
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=> s restricti?(s)endonucleas?(s)type?(s)ii
L2        4500 RESTRICTI?(S) ENDONUCLEAS?(S) TYPE?(S) II

=> s l2(s)(iig? or ii(4w)g?)
TERM 'G?' EXCEEDED TRUNCATION LIMITS - SEARCH ENDED
You have entered a truncated stem which occurs in too many terms.
Make the stem longer and try again.  For example, if your original
term was 'degr?' to search for variations and the abbreviation for
'degradation', you could replace it with the expression '(degrdn OR
degrad?)'.  If your search term was numeric, e.g., 'C>5', reduce the
size of the range.

=> s l2(s)(iig? or ii(4w)g)
L3        366 L2(S)(IIG? OR II(4W) G)

=> s l3(s)(chimer? or muta? or combin? or fus?)
   9 FILES SEARCHED...
L4        331 L3(S)(CHIMER? OR MUTA? OR COMBIN? OR FUS?)

=> dup rem l4
DUPLICATE IS NOT AVAILABLE IN 'GENBANK'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L4
L5         329 DUP REM L4 (2 DUPLICATES REMOVED)

=> s l5(s)(cleav? or cataly? or methylas? or targe? or specif? or recogn? or bind?)
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L38(S)(CLEAV?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L44(S)(CLEAV?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L46(S)(CLEAV?'
   6 FILES SEARCHED...
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L48(S)(CLEAV?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L50(S)(CLEAV?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L52(S)(CLEAV?'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L54(S)(CLEAV?'
L6        329 L5(S)(CLEAV? OR CATALY? OR METHYLAS? OR TARGE? OR SPECIF? OR
              RECOGN? OR BIND?)
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```
=> focus l6
FOCUS NOT AVAILABLE IN 'GENBANK'.
PROCESSING COMPLETED FOR L6
ANSWERS FROM NON FOCUS FILES PUT AT END OF ANSWER SET.
L7          329 FOCUS L6 1-

=> d ti l7 1-100

L7    ANSWER 1 OF 329  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI    New pure Type IIG restriction endonuclease obtainable from Citrobacter
      species or from Escherichia coli, useful for generating restriction
      endonucleases with new specificities;
         for use in genetic engineering

L7   ANSWER 2 OF 329  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of pancreatic
       cancer

L7   ANSWER 3 OF 329  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of colon cancer

L7   ANSWER 4 OF 329  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of ovarian cancer

L7   ANSWER 5 OF 329  USPATFULL on STN
TI     Compositions and methods for the therapy and diagnosis of colon cancer

L7   ANSWER 6 OF 329  USPATFULL on STN
TI     Methods for altering the cleavage specificity of a type IIG restriction
       endonuclease

L7    ANSWER 7 OF 329  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI    Novel human alpha-hydroxysteroid dehydrogenase-like enzyme useful for
      treating, preventing and ameliorating cancer, glaucoma, obesity, colon
      and prostate cancer, and benign prostate hypertrophy;
         fusion protein, drug screening, agonist, antagonist, antibody,
         antisense and ribozyme useful for gene therapy

L7   ANSWER 8 OF 329      GENBANK®  COPYRIGHT 2007 on STN

   TITLE (TI):          Complete sequence of Marinomonas sp. MWYL1
   TITLE (TI):          Direct Submission

L7   ANSWER 9 OF 329      GENBANK®  COPYRIGHT 2007 on STN

   TITLE (TI):          Complete sequence of Actinobacillus succinogenes 130Z
   TITLE (TI):          Direct Submission

L7   ANSWER 10 OF 329     GENBANK®  COPYRIGHT 2007 on STN

   TITLE (TI):          Direct Submission

L7   ANSWER 11 OF 329     GENBANK®  COPYRIGHT 2007 on STN

   TITLE (TI):          Direct Submission

L7   ANSWER 12 OF 329     GENBANK®  COPYRIGHT 2007 on STN

   TITLE (TI):          Complete genome sequencing of Staphylococcus aureus
                        strain Newman and its comparative analysis with other
                        S. aureus genomes
   TITLE (TI):          Direct Submission

L7   ANSWER 13 OF 329     GENBANK®  COPYRIGHT 2007 on STN
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TITLE (TI): Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori
TITLE (TI): Direct Submission

L7 ANSWER 324 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon Archaeoglobus fulgidus
TITLE (TI): Direct Submission

L7 ANSWER 325 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): Genome sequence of the radioresistant bacterium Deinococcus radiodurans R1
TITLE (TI): Direct Submission

L7 ANSWER 326 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): The complete genome sequence of the gastric pathogen Helicobacter pylori
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission

L7 ANSWER 327 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): Complete genome sequence of the methanogenic archaeon, Methanococcus jannaschii
TITLE (TI): Direct Submission

L7 ANSWER 328 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): Whole-genome random sequencing and assembly of Haemophilus influenzae Rd
TITLE (TI): Metabolism and evolution of Haemophilus influenzae deduced from a whole-genome comparison with Escherichia coli
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission

L7 ANSWER 329 OF 329 GENBANK® COPYRIGHT 2007 on STN

TITLE (TI): The complete genome sequence of Escherichia coli K-12
TITLE (TI): Escherichia coli K-12: a cooperatively developed annotation snapshot--2005
TITLE (TI): Workshop on Annotation of Escherichia coli K-12
TITLE (TI): ASAP: Escherichia coli K-12 strain MG1655 version m56
TITLE (TI): A more accurate sequence comparison between genomes of Escherichia coli K12 W3110 and MG1655 strains
TITLE (TI): Escherichia coli K-12 MG1655 yqiK-rfaE intergenic region, genomic sequence correction
TITLE (TI): A manual approach to accurate translation start site annotation: an E. coli K-12 case study
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission
TITLE (TI): Direct Submission

=> d l7 ibib abs 1 2 6

L7 ANSWER 1 OF 329 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-29788 BIOTECHDS

TITLE: New pure Type IIG restriction endonuclease obtainable from Citrobacter species or from Escherichia coli, useful for generating restriction endonucleases with new specificities; for use in genetic engineering

AUTHOR: MORGAN R; WILSON G; LUNNEN K; HEITER D; BENNER J; NKENFOU C N; PICONE S

PATENT ASSIGNEE: NEW ENGLAND BIOLABS INC

PATENT INFO: WO 2005094516 13 Oct 2005

APPLICATION INFO: WO 2005-US9824 23 Mar 2005

PRIORITY INFO: US 2004-555796 24 Mar 2004; US 2004-555796 24 Mar 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-714328 [73]

AN 2005-29788 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A substantially pure Type IIG restriction endonuclease (I) obtainable from Citrobacter sp. 2144 (NEB#1398) (American Type Culture Collection (ATCC) Patent Accession Number PTA-5846) or from Escherichia coli NEB#1554 (ATCC Patent Accession Number PTA-5887), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated DNA obtainable from Citrobacter sp. 2144 (NEB (2) 1398) (ATCC Patent Accession Number PTA-5846) or from E.coli NEB (3) 1554 (ATCC Patent Accession Number PTA-5887) and encoding (I), where the DNA comprises a first DNA segment expressing an endonuclease and methyl transferase catalytic function and a second DNA segment encoding a sequence specificity function of the restriction endonuclease, where the first and second DNA segments comprise one or more DNA molecules; (4) a recombinant DNA vector comprising at least one of first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease; (5) a host cell (II) transformed with a first DNA segment coding for the restriction and modification domains of CspCI restriction endonuclease and a second segment coding for the specificity domain of the restriction endonuclease, where the first DNA segment and the second DNA segment are contained with one or more DNA vectors; (6) preparing (I); and (7) making (M1) Type II restriction endonuclease having an altered specificity comprising: (a) selecting a restriction endonuclease from a set of enzymes, where each enzyme in the set is characterized by a modular structure having a specificity subunit and a catalytic subunit, the specificity subunit further comprising N-terminal domain for binding one half site of a bipartite recognition sequence and a C-terminal domain for binding a second half site of the bipartite recognition sequence; (b) modifying the specificity subunit; and (c) obtaining the Type II restriction endonuclease with altered specificity.

BIOTECHNOLOGY - Preparation: Preparing (I), involves cultivating a sample of Citrobacter sp. 2144 (NEB#1398) or (II) under conditions favoring the production of the endonuclease, and purifying the endonuclease (claimed). Preferred Endonuclease: (I) Is capable of recognizing at least one sequence chosen (SEQ ID No: 32-35), and cleaving the DNA on both sides of the recognition sequence. Preferred Method: In (M1), modifying the specificity subunit further comprises: (a) substituting the N-terminal domain with a second C-terminal domain or substituting the C-terminal domain with a second N-terminal domain; (b) substituting the N-terminal domain or the C-terminal domain or both N-terminal and C-terminal domain with a binding domain from a second restriction endonuclease or methyltransferase;

(c) mutating the N-terminal domain, the C-terminal domain or both domains to alter the binding specificity; or (d) changing the length of the spacer amino acid sequence between the N-terminal and C-terminal domains of the specificity module. The second restriction endonuclease or methyltransferase is chosen from Type I restriction endonuclease, Type IIG restriction endonuclease and gamma-type m6A methyltransferase. The specificity subunit and the catalytic subunit are encoded by different genes. (I) Comprises sequences such as nnnnnnnnnncaannnnngtggnnnnnnnnnnnnn (SEQ ID No: 32), nnnnnnnnnnncaannnnngtggnnnnnnnnnnnn (SEQ ID No: 33), caannnnngtgg (SEQ ID No: 34), caannnngtgg (SEQ ID No: 35), where n=a, c, t or g.

USE - (I) Is useful for generating endonucleases with new specificity, for innovative genetic engineering.

EXAMPLE - CspCI was obtained by culturing either Citrobacter sp. 2144 (NEB#1398) or the transformed host Escherichia coli NEB#1554, and recovering the endonuclease from the cells. Citrobacter sp. 2144 (NEB#1398) or E.coli NEB#1554 were incubated aerobically at 37degreesC. Cells in the late logarithmic stage of growth were collected by centrifugation and either disrupted immediately or stored frozen at -70degreesC. The cell paste was suspended in a buffer solution and ruptured by sonication, high pressure dispersion or enzymatic digestion to allow extraction of the endonuclease by the buffer solution. Intact cells and cellular debris were then removed by centrifugation to produce a cell-free extract containing CspCI. The CspCI endonuclease was then purified from the cell-free extract by ion exchange chromatography, affinity chromatography, molecular sieve chromatography, or their combinations. 277 grams of E.coli NEB#1554 CspCI cell pellet or Citrobacter sp. 2144 were suspended in 1 liter of buffer A containing 300mM sodium chloride, and passed through a Gaulin homogenizer at 12000 psig. The lysate was centrifuged at 13000xG for 40 minutes and the supernatant collected. The supernatant solution was applied to a 400 ml diethylaminoethyl (DEAE) fast flow column. The diluted enzyme was applied to a 375 ml heparin hyper D column. A 2.5 L wash of buffer B was applied, then a 2 L gradient of sodium chloride from 0.15-1M in buffer B was applied and fractions were collected. Fractions were assayed for CspCI endonuclease activity by incubating with 1 microgram of phase lambda DNA (NEB) in 50 mulNEB buffer 2, supplemented with 20 micromolar for 15 minutes at 37degreesC. CspCI activity eluted at 0.3-0.35 M sodium chloride. CspCI activity eluted at 0.4-0.5 M potassium hydrogen phosphate. (87 pages)

L7 ANSWER 2 OF 329 USPATFULL on STN

ACCESSION NUMBER: 2003:106233 USPATFULL

TITLE: Compositions and methods for the therapy and diagnosis of pancreatic cancer

INVENTOR(S): Benson, Darin R., Seattle, WA, UNITED STATES
Kalos, Michael D., Seattle, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Persing, David H., Redmond, WA, UNITED STATES
Hepler, William T., Seattle, WA, UNITED STATES
Jiang, Yuqiu, Kent, WA, UNITED STATES

PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 2003073144 | A1 | 20030417 | |
| APPLICATION INFO.: | US 2002-60036 | A1 | 20020130 | (10) |

| | NUMBER | DATE | |
|---|---|---|---|
| PRIORITY INFORMATION: | US 2001-333626P | 20011127 | (60) |
| | US 2001-305484P | 20010712 | (60) |

| | |
|---|---|
| | US 2001-265305P 20010130 (60) |
| | US 2001-267568P 20010209 (60) |
| | US 2001-313999P 20010820 (60) |
| | US 2001-291631P 20010516 (60) |
| | US 2001-287112P 20010428 (60) |
| | US 2001-278651P 20010321 (60) |
| | US 2001-265682P 20010131 (60) |
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | APPLICATION |
| LEGAL REPRESENTATIVE: | SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092 |
| NUMBER OF CLAIMS: | 17 |
| EXEMPLARY CLAIM: | 1 |
| LINE COUNT: | 14253 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for the therapy and diagnosis of cancer, particularly pancreatic cancer, are disclosed. Illustrative compositions comprise one or more pancreatic tumor polypeptides, immunogenic portions thereof, polynucleotides that encode such polypeptides, antigen presenting cell that expresses such polypeptides, and T cells that are specific for cells expressing such polypeptides. The disclosed compositions are useful, for example, in the diagnosis, prevention and/or treatment of diseases, particularly pancreatic cancer.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 6 OF 329 USPATFULL on STN

| | |
|---|---|
| ACCESSION NUMBER: | 2004:280284 USPATFULL |
| TITLE: | Methods for altering the cleavage specificity of a type IIG restriction endonuclease |
| INVENTOR(S): | Xu, Shuang-yong, Lexington, MA, UNITED STATES<br>Kobbe, Daniela, Karlsruhe, GERMANY, FEDERAL REPUBLIC OF<br>Zhu, Zhenyu, Beverly, MA, UNITED STATES<br>Samuelson, James, Danvers, MA, UNITED STATES |

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 2004219584 | A1 | 20041104 |
| APPLICATION INFO.: | US 2004-800946 | A1 | 20040315 (10) |

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|---|---|
| RELATED APPLN. INFO.: | Continuation-in-part of Ser. No. US 2002-150028, filed on 17 May 2002, ABANDONED Division of Ser. No. US 2000-693146, filed on 20 Oct 2000, GRANTED, Pat. No. US 6413758 |
| DOCUMENT TYPE: | Utility |
| FILE SEGMENT: | APPLICATION |
| LEGAL REPRESENTATIVE: | NEW ENGLAND BIOLABS, INC., 32 TOZER ROAD, BEVERLY, MA, 01915 |
| NUMBER OF CLAIMS: | 16 |
| EXEMPLARY CLAIM: | 1 |
| NUMBER OF DRAWINGS: | 11 Drawing Page(s) |
| LINE COUNT: | 1472 |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods are provided for altering the cleavage specificity of a Type IIG restriction endonuclease, the Type IIG restriction endonuclease being characterized by a cleavage domain adjacent to a methylase domain, the methylase domain located adjacent to a specificity domain. The method includes ligating DNA or protein sequences to form a fusion DNA or fusion protein. Where a fusion DNA is formed, the host cell is transformed with the fusion DNA to express a Type IIG restriction endonuclease with altered cleavage specificity.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> s l7 and (bmpi? or acui? or thaiv? or bsgi?)

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L8            1 L7 AND (BMPI? OR ACUI? OR THAIV? OR BSGI?)

=> d ti l8

L8   ANSWER 1 OF 1  USPATFULL on STN
TI     Methods for altering the cleavage specificity of a type IIG restriction
       endonuclease


=> s (bmpi? or acui? or thaiv? or bsgi?)
L9       165765 (BMPI? OR ACUI? OR THAIV? OR BSGI?)

=> s l9(s)(methyl? or catalyti? or specifi? or bind? or recogn?)
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   9 FILES SEARCHED...
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=> s l10(s)(chime? or fus? or muta? or combin?)
L11         381 L10(S)(CHIME? OR FUS? OR MUTA? OR COMBIN?)

=> s l11(s)(iig or ii(2w)g)
L12           3 L11(S)(IIG OR II(2W) G)

=> d ti l12 1-3

L12  ANSWER 1 OF 3  USPATFULL on STN
TI     Methods for altering the cleavage specificity of a type IIG restriction
       endonuclease

L12   ANSWER 2 OF 3  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI    Preparing DNA fragments comprising sequences corresponding to two
      opposite end regions of linear nucleic acid, for e.g. analysis, comprises
      ligating linkers, circularizing, and digesting;
         DNA fragment preparation for use in cancatemer and expression
         profiling

L12   ANSWER 3 OF 3  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
TI    Preparing DNA fragment corresponding to nucleotide sequence of 5' end
      region of mRNA, by preparing nucleic acid corresponding to nucleotide
      sequence of 5' end of mRNA, cleaving nucleic acid with restriction
      enzyme;
         for use in diagnosis


=> d ibib abs l12 1-3

L12  ANSWER 1 OF 3  USPATFULL on STN
ACCESSION NUMBER:        2004:280284  USPATFULL
TITLE:                   Methods for altering the cleavage specificity of a type
                         IIG restriction endonuclease
INVENTOR(S):             Xu, Shuang-yong, Lexington, MA, UNITED STATES
                         Kobbe, Daniela, Karlsruhe, GERMANY, FEDERAL REPUBLIC OF
                         Zhu, Zhenyu, Beverly, MA, UNITED STATES
                         Samuelson, James, Danvers, MA, UNITED STATES

                              NUMBER         KIND    DATE
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PATENT INFORMATION:      US 2004219584      A1   20041104
APPLICATION INFO.:       US 2004-800946     A1   20040315  (10)
RELATED APPLN. INFO.:    Continuation-in-part of Ser. No. US 2002-150028, filed
                         on 17 May 2002, ABANDONED Division of Ser. No. US
                         2000-693146, filed on 20 Oct 2000, GRANTED, Pat. No. US
                         6413758
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
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NUMBER OF CLAIMS: 16
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L12 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-04877 BIOTECHDS
TITLE: Preparing DNA fragments comprising sequences corresponding to two opposite end regions of linear nucleic acid, for e.g. analysis, comprises ligating linkers, circularizing, and digesting;
DNA fragment preparation for use in cancatemer and expression profiling
AUTHOR: HARBERS M; SHIBATA Y
PATENT ASSIGNEE: DNAFORM KK
PATENT INFO: WO 2006003721 12 Jan 2006
APPLICATION INFO: WO 2004-JP9862 2 Jul 2004
PRIORITY INFO: WO 2004-JP9862 2 Jul 2004; WO 2004-JP9862 2 Jul 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2006-100543 [10]
AN 2006-04877 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Preparing DNA fragments comprising sequences corresponding to two opposite end regions of a linear nucleic acid molecule, comprises creating a linear DNA molecule from a nucleic acid molecule, ligating linkers to two opposite ends of the linear DNA molecule, circularizing the linear DNA molecule, digesting the circular DNA molecule with a restriction endonuclease, and isolating the DNA fragment.

DETAILED DESCRIPTION - Preparing (M1) DNA fragments comprising sequences corresponding to two opposite end regions of a linear nucleic acid molecule, comprises: (a) creating a linear DNA molecule from a nucleic acid molecule; (b) ligating linkers to two opposite ends of the linear DNA molecule, where the linkers contain a cloning site and a recognition site for a restriction endonuclease that cleaves at a site outside its recognition site and within the linear DNA molecule; (c) circularizing the linear DNA molecule by closing the linear DNA molecule at its cloning site so as to form a circular DNA molecule; (d) digesting the circular DNA molecule with a restriction endonuclease that cleaves at a site outside its recognition site and cuts out a DNA fragment from the circular DNA molecule, where the DNA fragment comprises opposite end regions of the linear DNA molecule; and (e) isolating the DNA fragment. INDEPENDENT CLAIMS are also included for the following: (1) vector pGSC; (2) obtaining (M2) information on the end sequences of a liner nucleic acid molecule, comprising preparing DNA fragments by (M1), preparing a concatemer by ligating the DNA fragments with each other, and sequencing the concatemer so as to obtain information on the end sequences of the linear nucleic acid molecule; and (3) priming (M3) a reverse transcription reaction, comprising: (a) preparing a double-stranded linker having a single-stranded overhanging region, where the single-stranded overhanging region is complementary to the 3'-end sequence of the RNA; (b) hybridizing the single-stranded

overhanging region to the complementary 3'-end sequence of the RNA so as to ligate the double-stranded linker to the 3'-end of the RNA; and (c) letting the free 3'-end of the overhanging region of the linker prime a reverse transcription reaction over the RNA with a reverse transcriptase.

BIOTECHNOLOGY - Preferred Method: In (M1), the linear nucleic acid molecule is an RNA, e.g. mRNA, DNA e.g. cDNA or a genomic DNA. The step of creating a linear DNA molecule from the RNA comprises converting the RNA into a complementary DNA by the means of a reverse transcriptase and a primer. The primer contains a Class IIS or Class III recognition site for removing stretches of oligo-dT used in the priming of the reverse transcription reaction from the RNA, which is a poly-adenylated RNA. The linear nucleic acid molecule is an RNA, which does not have a poly-A tail at its 3' end. The step of creating a linear DNA molecule from a linear nucleic acid molecule involves preparing a double-stranded linker having a single-stranded overhanging region, where the single-stranded overhanging region is complementary to the 3'-end sequence of the RNA, hybridizing the single-stranded overhanging region to the complementary 3'-end sequence of the RNA so as to ligate the double-stranded linker to the 3'-end of the RNA, letting the free 3'-end of the overhanging region of the linker prime a reverse transcription reaction over the RNA with a reverse transcriptase, and separating a linear DNA molecule from the reverse transcription product. The linear DNA molecule prepared from the RNA is enriched by the means of the cap-structure in the RNA. The enrichment is performed by captrapping, oligo-capping, or a substance specifically binding to the cap structure of the RNA. The complementary sequences derived from a poly-A tail of the mRNA are removed from the linear cDNA molecule. The cDNA is a full-length cDNA. The restriction enzyme that cleaves at a site outside its recognition site is chosen from Class IIS and Class IIG restriction enzymes GsuI, MmeI, BpmI, BsgI or any of their mixture. The restriction enzyme that cleaves at a site outside its recognition site is the Class III restriction enzyme EcoR15I or a mixture containing EcoR15I. The linkers are attached to a selective binding substance to allow for enrichment by such binding. The selecting binding substance is chosen from biotin and digoxigenin, and the high affinity binding substance is chosen from avidin, streptavidin, derivative of avidin or streptavidin, and an anti-digoxigenin antibody. The linkers contain sequence elements used for labeling the DNA fragment. The label is composed of a short sequence of 4-12 base pairs in length. The label comprises the recognition site for a restriction endonuclease or a recombinase. (M1) Further comprises ligating or combining the linear DNA molecule to form a circularized DNA molecule. The circularization step is performed by the means of a ligation reaction or a recombinase. The linear DNA fragments are removed from the circular DNA molecule by the means of an exonuclease which is exonuclease III, exonuclease I, or any of its mixture. (M1) Further involves amplifying the circular DNA molecule by the means of a rolling circle reaction. The amplification makes use of random priming and Phi29 DNA polymerase. The circular DNA molecule is cut by one or more restriction enzyme that cleaves at a site outside its recognition site. The DNA fragment that is cut out by the means of the restriction enzyme and that comprises the cloning site used in the circularization step and comprises opposite end regions of the linear DNA molecule is separated from the remaining part of the DNA molecule lacking the end regions. In (M2), the DNA fragment is derived from a mixed sample. The origin of the DNA fragment in the mixed sample can be tracked by a label, which is a shot specific sequence in the spacer. In (M3), the overhanging part of the linker is comprised of oligo-dT. The 3'-end of the oligo-dT overhang is blocked. The linker is attached to a selective binding substance used for the fractionation of RNAs. (M3) Further involves attaching the linker to a high affinity selective binding substance so as to allow for enrichment.

USE - (M1) Is useful for preparing DNA fragments comprising sequences corresponding to two opposite end regions of a linear nucleic

acid molecule. (M1) Is useful for preparing a concatemer, which involves ligating the DNA fragments obtained by (M1) to each other so as to form a concatemer, and ligating the concatemer into a vector, e.g. pGSC. (M1) Is useful for obtaining information on the end sequences of a liner nucleic acid molecule (all claimed). (M1) Is useful for analysis of fragments for the purpose of gene identification and expression profiling and for studies on biological system, characterization of genetic elements and analysis the expressed genes. The identified DNA fragments are useful in drug development, diagnostics or forensic studies.

EXAMPLE - No relevant example is given. (70 pages)

L12 ANSWER 3 OF 3 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-06620 BIOTECHDS
TITLE: Preparing DNA fragment corresponding to nucleotide sequence of 5' end region of mRNA, by preparing nucleic acid corresponding to nucleotide sequence of 5' end of mRNA, cleaving nucleic acid with restriction enzyme; for use in diagnosis
AUTHOR: HAYASHIZAKI Y; CARNINCI P; HARBERS M T
PATENT ASSIGNEE: RIKEN KK; DNAFORM KK
PATENT INFO: WO 2003106672 24 Dec 2003
APPLICATION INFO: WO 2003-JP7514 12 Jun 2003
PRIORITY INFO: JP 2002-235294 12 Aug 2002; JP 2002-171851 12 Jun 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-082194 [08]
AN 2004-06620 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Preparing (M1) a DNA fragment (I) corresponding to a nucleotide sequence of a 5' end region of an mRNA, comprising preparing a nucleic acid (II) corresponding to a nucleotide sequence of the 5' end of an mRNA, attaching a linker to (II), cleaving (II) with a restriction enzyme having its recognition site within the linker and collecting a resulting (I) corresponding to the 5' end of the mRNA, is new.

DETAILED DESCRIPTION - Preparing (M1) a DNA fragment (I) corresponding to a nucleotide sequence of a 5' end region of an mRNA, comprises: (a) preparing a nucleic acid (II) corresponding to a nucleotide sequence of the 5' end of an mRNA, attaching a linker to (II), cleaving (II) with a restriction enzyme having its recognition site within the linker and its cleavage site within (II) corresponding to the 5' end of the mRNA and collecting a resulting (I) corresponding to the 5' end of the mRNA; or (b) substituting a cap structure of an mRNA with an oligonucleotide, synthesizing a first strand cDNA using the mRNA as a template, synthesizing a second strand cDNA using the first stand cDNA as a template, cleaving a resulting double stranded cDNA with the restriction enzyme and collecting a resulting (I), where the oligonucleotide comprises a restriction enzyme recognition site, and a cleavage site of a restriction enzyme in (II). INDEPENDENT CLAIMS are also included for: (1) a concatemer (III) prepared by (M1); (2) a vector comprising (III); and (3) a sequence derived from (III).

BIOTECHNOLOGY - Preferred Method: (M1) further involves amplifying the nucleic acid corresponding the 5' end region of the mRNA by a DNA polymerase or a cocktail of DNA polymerases, attaching the collected nucleic acid to beads and extending the 5' end region of the nucleotide sequence, where the DNA polymerase is heat-stable. In (M1), the length of (I) is 5-100 bp, preferably 15-30 bp and most preferably 10-30 bp. (II) is derived from a total RNA, an mRNA, full-length cDNA, a biological sample, an in vitro synthesized RNA, a cDNA library, artificially created several of nucleic acids, or a tag library. The preparing step of (a) involves substituting a 5' cap structure of the mRNA with an oligonucleotide and synthesizing a first-strand cDNA using the mRNA as a template to produce (II). The preparing step of (a) further involves synthesizing first-strand cDNAs using RNAs as a template and producing cDNA/RNA hybrids of the resulting first-strand cDNAs and RNAs, selecting a particular cDNA/RNA hybrid that has the 5' cap structure of the mRNA

using a selective binding substance which specifically recognizes the 5' cap structure, and recovering (II), or conjugating selective binding substance (IV) to a 5' cap structure of an mRNA present in the RNAs, contacting the cDNA/RNA hybrids with a support, and recovering (II) from the mRNA fixed to the support, where another matching selective binding substance is fixed to the support, and the matching selective binding substance specifically binds to (IV). (II) is a full-length cDNA, where (IV) is attached to a support. (IV) is a cap binding protein or a cap binding antibody such as digoxigenin. (IV) is biotin, and the matching selective binding substance is chosen from the group consisting of avidin, streptavidin and a derivative which specifically binds to biotin, such as antibody against digoxigenin. The support is made of magnetic beads, agarose beads, latex beads, sepharose matrix, silica gel matrix or glass beads. The attaching step of (a) involves attaching a linker to an end region corresponding to the nucleotide sequence of a 5' end region of the mRNA, synthesizing a second-strand cDNA (V) using (II) as a template, treating a resulting linker-bound double-strand cDNA with the restriction enzyme and recovering a resulting fragment which contains a linker moiety and a part of cDNA corresponding to the 5' end regions of the mRNA, where the linker carries a restriction enzyme recognition site for a restriction enzyme that cleaves a site different from its recognition sequence. The linker contains a double-stranded oligonucleotide region, and (V) is synthesized using the linker or other oligonucleotides which are partially or totally complement to the linker. (IV) is attached to or included in the linker, and the recovering step involves the steps of binding (IV) to a matching selective binding substance immobilized on a support, and recovering the support, where the matching selective binding substance specifically binds to (IV). The restriction enzyme is the Class II or Class III restriction enzyme, preferably class IIG and Class IIS restriction enzymes. The restriction enzyme is chosen from GsuI, MmeII, BpmI, BsgI or EcoP15I. The DNA polymerase is chosen from Taq polymerase, Pwo DNA polymerase, Kod DNA polymerase, Pfu DNA polymerase, Vent DNA polymerase, Deep Vent DNA polymerase, rBST DNA polymerase, and Master Amp AmpliTherm DNA polymerase. The first strand cDNA of (a) is synthesized and fractionated by physical means. (II) is fractionated by hybridizing to several of nucleic acids.

USE - (M1) is useful for the development of diagnostic tools, research tools and a reagent or a kit. (M1) is useful for preparing (I) corresponding to a nucleotide sequence of a 5' end region of an mRNA. (I) prepared by (M1) is useful for determining a nucleotide sequence of the 5' region of the mRNA which involves sequencing (I). (I) prepared by (M1) is useful for preparing a concatemer comprising one or more DNA fragments which involves ligating one or more of (I) that corresponds to the 5' end of the mRNA. (I) is useful for determining the transcriptional states of a sample, obtaining and quantifying expression data on a several of mRNAs or cDNAs in a sample, building a database holding sequence information, identifying transcribed regions from a genomic sequence and identifying a transcription initiation site and a related regulatory sequence in a genomic sequence, by a sequence derived from (I). (I) prepared by (M1) is useful for cloning a full-length or partial cDNA from a cDNA library or biological sample, cloning a complete or partial promoter region of a gene from a genomic library or genomic DNA, analyzing the activity of regulatory regions in a genome based on genomic sequence information and inactivating a gene or altering its expression, by using a sequence derived from (I), where the gene is inactivated or altered in its expression by the means of siRNA or RNA. (I) is useful for synthesizing a nucleotide sequence to be used as the linker or primer, and a hybridization on probe based on a sequence derived from (I), where the hybridization probe is attached to a support. The hybridization probe is a probe to identify the sequence corresponding to the nucleotide sequence of the 5' end region of the mRNA (all claimed). (M1) is useful for selectively collecting multiple nucleic acid fragments containing

information on the nucleotide sequences at the 5' end of multiple mRNA in a sample, analyzing complex regulatory networks in combination with the ability to identify and clone new genes opens a wide area of applications for monitoring biological systems and their status in development, homeostasis disease, and for identifying differentially expressed genes. (III) is useful for identifying regions in the genome, which are required for gene regulation and gene expression.

EXAMPLE - All mRNA samples were analyzed for their ratios of the OD readings at 230, 260 and 280 nm to monitor the mRNA purity. The first-strand cDNA was prepared from different mRNA samples using Superscript II and the purified cDNA primer such as 5'-(ga)5aaggatcctgccatttcattacctctttctccgcacccgacataga(t)16vn'-3'. The full-length cDNAs were isolated using magnetic beads coated with streptavidin. The double-stranded linker was assembled out of two upper strand oligonucleotides with random overhangs and a shorter lower strand oligonucleotide. For ligation of the linker to the single-stranded cDNA, 2 microg of linker per 1 microg cDNA were used. In a final volume of 7.5 microl of 0.1xTE, the cDNA and the linker were mixed and incubated at 65 degrees Centigrade for 5 minutes to melt secondary structures in the cDNA. The double-stranded linker was then ligated to the single-stranded cDNA using a TAKaRa ligation kit, version 2. The ligation reaction was terminated by adding 1 microl of 0.5 M ethylenediamine tetraacetic acid (EDTA), 1 microl of 10% sodium dodecyl sulfate (SDS), 1 microl of 10 mg/ml proteinase K, and 10 microl of water. After incubation at 45 degrees Centigrade for 15 minutes the resulting mixture was extracted with the three-fold excess of Tris-equilibrated phenol/chloroform. The remaining excess of free linker was removed from the reaction mixture by gel filtration of the solution in a S-300 spin column. Briefly, the S-300 columns were transferred into a centrifugation tube and spun at 3,000 rpm for 1 minutes to remove the storage buffer from the column. After placing the column in a new centrifugation tube the DNA sample followed by another 40 microl of water were added to the column and the column was spun with 3,000 rpm for 5 minutes at 4 degrees Centigrade to collect the run through. To concentrate the DNA the eluate from the S300 column was placed on Microcon 100 membrane and centrifuged until a final volume of 10 lambda was achieved. The membrane was washed once with 10 microl of 0.1xTE at 65 degrees Centigrade for 3 minutes and the fractions were united for use in the following second strand synthesis. The second strand cDNA was synthesized 5'-Bio-agagagagacctcgagtaactataacggtcctaaggta gcgacctaggtccgacg-3'. The resulting double-stranded cDNA was cleaved with a Class IIS restriction enzyme, MmeI. After having cleaved the double-stranded cDNA with the Class IIS restriction enzyme MmeI a second linker was ligated to the 2 bp overhang at the cleavage site. Ligation products having biotin moistures at the 5' end were separated from none modified DNA, using streptavidin coated magnetic beads. DNA fragments bound to the magnetic beads by the means of a biotin-streptavidin interaction were released from the beads by treatment with an excess of free biotin. The DNA was further purified by gel filtration on a G50 spun column. Before cloning the DNA fragments were amplified by a PCR and re-amplified by a second PCR. The purified PCR product was digested by the restriction enzymes XmaJI and XbaI. The resulting 33 bp DNA fragments were separated from the free DNA ends cut off during the restriction digests by incubation with streptavidin coated magnetic beads, which would retain the biotin-labeled DNA fragments. The DNA was further purified by RNaseI, proteinase K treatment and 12% polyacrylamide gel. DNA fragments comprising 5'ends were ligated with each other to form concatemers. Thus the DNA fragment corresponding to a nucleotide sequence of a 5' end region of an mRNA was obtained. (121 pages)

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      HIGHEST APPLICATION PUBLICATION NUMBER: US2007157352
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